August 15, 2002

Timothy Adams, Ph.D.
Technical Contact
The Flavor and Fragrance High Production
Volume Consortia
1620 I Street, N.W.
Washington, D.C. 20006

Dear Dr. Adams:

The Office of Pollution and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Ionone Derivatives category posted on the ChemRTK HPV Challenge Program Web site on April 19, 2002. I commend The Flavor and Fragrance High Production Volume Consortia for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed Comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The Flavor and Fragrance High Production Volume Consortia advise the Agency, within 90 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the HPV Challenge Program Web site "Submit Technical Questions" button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-S-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: W. Sanders
A. Abramson

C. Auer
M. E. Weber

**EPA Comments on Chemical RTK HPV Challenge Submission:
Ionone Derivatives Category**

SUMMARY OF EPA COMMENTS

The sponsor, the Terpene Consortium of The Flavor and Fragrance High Production Volume Consortia, submitted a test plan and robust summaries to EPA for the Ionone Derivatives category dated March 27, 2002. EPA posted the submission on the ChemRTK HPV Challenge Web site on April 19, 2002.

EPA has reviewed this submission and has reached the following conclusions:

1. Category Justification. The submitter's support for grouping the chemicals under this category is acceptable. However, the submitter needs to clarify how data on related chemicals will be used to support the proposed category.

2. Physicochemical Properties and Environmental Fate. The submitter needs to address the melting point and water solubility endpoints. The treatment of the stability in water endpoint needs to be clarified. All other endpoints are adequately addressed.

3. Health Effects. EPA agrees that the data can be reasonably extrapolated between the category members. However, the submitter needs to revise the test plan table to indicate the use of data on related chemicals. The developmental toxicity endpoint has not been adequately addressed. Under the HPV Challenge Program a developmental screening test would provide the necessary data.

4. Ecological Effects. EPA agrees that testing is necessary for the ionone derivatives but has reservations about the test plan. (a) EPA prefers that the acute daphnid test be conducted on methylionone isomers instead of *alpha-iso*-methylionone. Any observed excess toxicity associated with methylionone's less substituted vinyl ketone moiety would trigger additional acute fish testing on methylionone. (b) The submitter needs to consider conducting a chronic daphnid test because there is a concern for both acute and chronic toxicity.

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

EPA COMMENTS ON THE IONONE DERIVATIVES CATEGORY CHALLENGE SUBMISSION

Category Definition

The submitter has proposed a category covering methylionone (mixed isomers, CAS No. 1335-46-2) and *alpha-iso*-methylionone (CAS No. 127-51-5). Ionone, the parent terpene, occurs in nature as three isomers (*alpha*, *beta*, and *gamma*) that differ in the position of one double bond. Methylionone has the same double bond isomers as ionone and a methyl group at the terminal position of the butanone side chain. There are contradictory descriptions of this mixture in the test plan. It is identified as both methyl- and isomethylionone isomers in the "identity of substances" section (page 1) but the "structural classification" section (page 6) states that the mixture contains only the three isomers of methylionone. EPA assumes the latter statement to be correct but recommends the ambiguity be addressed.

Iso-methylionone has the same double bond isomers as ionone and a methyl group at the 2-position of the butanone side chain; only the *alpha* isomer is a category member.

The structure presented for *alpha*-ionone in the test plan is identical with that presented for *beta*-ionone and all its metabolites in Figure 1. The error needs correction.

Category Justification

The submitter bases the category on similar chemical composition and metabolism. Chemically, the only structural difference between *alpha-iso*-methylionone and the methylionone mixture is that *alpha-iso*-methylionone contains an additional methyl group at the 2-position of alpha-ionone while methylionone contains an additional methyl group at the 4-position of alpha-ionone. Orally administered ionones are absorbed and metabolized in mammals by allylic hydroxylation of the ring followed by oxidation of the hydroxyl group to 3-oxo derivatives Reduction of the ketone function to the corresponding secondary alcohol also occurs. Combinations of these detoxication reactions result in the formation of multiple polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acids. The metabolism of ionones is expected to be similar in humans. This is supported by human metabolism studies of retinoids and carotenoids that possess ionone fragments.

Although the submitter did not summarize the existing data on the category members and the parent ionones, the data are reasonably concordant which strengthens the justification for the proposed category. Based on the close structural similarity and expected similar metabolism, EPA agrees that the existing testing can be reasonably extrapolated to the category members for health. EPA believes that more testing is needed to determine if the same holds true for ecological effects.

As discussed below (see Test Plan comments), the submitter has not clearly explained how the data on the parent terpene ionone and other isomers are being used to support the proposed category.

A related concern is that the test plan refers to “pseudoionones” without providing structural information other than that they are open-chain synthetic precursors to ionones. This is significant because in the robust summaries data on pseudoionones are presented as analog data for category members, but without justification for an analogy between the two rather different structures. Similarly, data on *“delta*-methylionone” appear in the robust summaries, but the structure and reason for its inclusion are lacking.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, water solubility, and partition coefficient).

EPA agrees with the submitter that no further testing is necessary for boiling point, vapor pressure and log $K_{ow.}$

Melting point. Although the chemical is a liquid at room temperature, the melting point should be determined according to OECD Guideline 102. If the melting point is greater than 0 °C, then it should be reported.

Water solubility. Insufficient information was presented in the water solubility robust summary for the methyl ionone mixture; the measured value did not agree well with an estimated value or with a measured value for *alpha-iso*-methylionone.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity).

Adequate data are available for the biodegradation endpoint.

Photodegradation. The submitter supplied a calculated value for *alpha-iso*-methylionone and proposes to extrapolate it to methylionone. While the results of a calculation for methylionone are expected to be similar, EPA prefers that the calculation be provided rather than extrapolating from a calculated value.

Stability in water. On page 12 of the test plan the submitter states that hydrolysis is not possible for any of the members of the category. However, in the test plan table on page 21 a calculated value is indicated for *alpha-iso*-methylionone and a NA (not applicable) for methylionone. The robust summary shows calculated $t_{1/2}$ values from 9 to 169 hours. The submitter needs to address this inconsistency.

Fugacity. The submitter estimated the fugacity of these chemicals using a Level I EQC model. Although EPA had previously recommended the use of EQC Level I, this model is somewhat limited. EPA now recommends a level III analysis, which is more rigorous. The EQC and EPIWIN Level III models are acceptable.

Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

Test data are available for these endpoints from studies using ionone, methylionone, *alpha-iso*-methylionone, and other related isomers. However, the submitter needs to clarify how the data on the non-category members are being used. For instance, in the test plan table on page 22, the submitter indicates that the reproductive/developmental endpoints will be addressed by the "category approach" i.e., read across from other test data. But in the test plan text and table this is not articulated. For the endpoints in question, data on non-category members are merely summarized. It is left to the reader to make the proper connections. The submitter needs to revise the test plan table to indicate where the non-category member analog data will be used or revise the category to include the ionones.

EPA considers the developmental toxicity data inadequate and recommends that the submitter conduct a developmental toxicity screening test (OECD TG 421) on one of the category members.

Acute Toxicity. The submitter provided summaries on several isomers; however, none is complete and lack critical information. Considering the weight-of-evidence, EPA considers this endpoint addressed for the purposes of the HPV Challenge Program. However, the submitter needs to provide the missing information in robust summaries.

Repeated-dose Toxicity. Available data on *alpha-iso*-methylionone are not adequate for the purposes of HPV Challenge Program because the studies were conducted using only one dose level that was also a NOAEL.

Reproductive toxicity. The two generation reproductive toxicity study on methyl ionone (mixture of isomers) was not conducted according to the OECD guidelines and alone is not adequate to address the reproductive toxicity endpoint because only one dose level was tested that was also the NOAEL. However, examination of the sex organs in the available repeated-dose studies did not reveal any effects and taken together address this endpoint.

Developmental Toxicity. The developmental toxicity study is inadequate because pregnant females were dosed only once on the 8th day of pregnancy. There was no evidence of maternal or fetal toxicity at the highest dose tested. This endpoint has not been adequately addressed for the purposes of HPV Challenge Program.

Ecological Effects (fish, invertebrates, and algae). The submitter proposes to perform acute daphnia and algae testing on *alpha-iso*-methylionone and extrapolate the results to methylionone. However, there is

little information supporting the SAR model for chemicals of this type. EPA believes that methylionone may be the most toxic member owing to its less-substituted vinyl ketone moiety, and should be tested instead of *alpha-iso*-methylionone for the proposed endpoints. If the results of the daphnid acute test show an increase in toxicity beyond what the SAR model predicts, then an acute fish test on methylionone should be conducted.

The 48-hour fish test on *beta*-ionone is considered inadequate owing to the shorter than 96-hour required test duration.

The submitter also needs to consider performing a daphnid chronic test given that the measured log Kow for *alpha-iso*-methylionone of 4.6 suggests a concern for chronic toxicity. Generally, EPA would recommend the chronic testing instead of the acute tests, but in this case, because toxicity was seen in the fish acute studies using *alpha-iso*-methylionone, the acute endpoints need to be addressed. All testing should be done using measured concentrations to account for potential chemical losses over the duration of the test.

Specific Comments on the Robust Summaries

General comment

Though out the robust summaries the title substance is one of the two category members but often is not the substance tested. To avoid confusion it is preferable to title each summary with the name of the substance tested and then to identify it as an analog of a specific chemical.

Health Effects

Acute Toxicity. Most summaries lack the following information: dose levels (when more than one dose used), method description, animals sex, observation period, clinical signs, mortality per sex and per dose level.

Genetic Toxicity. The missing information in the Ames test is the number of dose levels, number of replicates, quantitative data, and explanation for not testing at the required concentrations and absence of cytotoxicity.

Ecotoxicity

Fish. A missing data element in the 96-hour study is water hardness.

Followup Activity

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.